EFFET OF ULTRAVIOLET RADIATION ON THE SURVIVAL OF THE DINOFLAGELLATE *PERIDINIUM BIPES* CAUSING FRESHWATER RED TIDE IN RESERVOIRS.

Zen'ichiro KAWABATA¹), Koichi SASAKI¹), Yasushi ISERI²), and Masaharu OCHA³)

Abstract

The dinoflagellate *Peridinium bipes* was irradiated with ultraviolet (UV) lamps which emit 253.7 nm light. The relationship between the time required for all the cells to die and the strength of continuous UV radiation was expressed by a hyperbolic curve, i.e., all the cells died by 6 hr and 48 hr under continuous UV radiation greater than 2400 and 80 $\mu W$ cm$^{-2}$, respectively. The time required for all the live cells to die after a certain of continuous UV radiation also conforms to a hyperbolic relation with strength of UV radiation, i.e., all the live cells were killed by UV radiation of 80 $\mu m$ cm$^{-2}$ for longer than 2 hr and 2400 $\mu m$ cm$^{-2}$ for longer than 1 min within 6 and 19 days, respectively.

Introduction

Dinoflagellates often cause water blooms, called freshwater red tides, in many reservoirs (Nakamoto, 1975; Herrgesell et al., 1976; Ito, 1979; Hata, 1983; Watanabe et al., 1983; Kagawa et al., 1984; Kawabata & Kagawa, 1988) and lakes (Horne et al., 1971; Serruya & Pollingher, 1971; Pollingher & Berman, 1975). Freshwater 'red tides' degrade water quality, create a nuisance in the filtration of drinking water, sometimes cause serious odour problems (Popovska, 1983), and rarely, mass mortality of fish (Hashimoto et al., 1968). Therefore, it is of importance for water quality management to suppress freshwater 'red tides'. Many strategies for mitigating eutrophication of lakes and reservoirs have been developed (Uhlmann, 1982; The Ministry of Construction, 1988; Kojima, 1988). There is yet, however, no effective method for suppression of freshwater 'red tides' caused by dinoflagellates because these organisms also grow in less eutrophicated water.

Ultraviolet (UV) radiation induces DNA damage (e.g., Levine et al., 1966; Kripke et al., 1983) and injures organisms. UV radiation, therefore,
has been widely used for sterilizing microorganisms. Recently UV radiation has been used for wastewater disinfection (WPCF Disinfection Committee, 1987). This suggests the possibility of applying UV radiation for the suppression of an increase in the population density or for elimination of the dinoflagellates causing freshwater 'red tides' in reservoirs. There is, however, been little quantitative investigation of the effect of UV radiation on the survival of the dinoflagellates.

The purpose of this laboratory study was to elucidate the effect of UV radiation on the survival of the dinoflagellate Peridinium bipes which causes a bloom in reservoirs (Watanabe et al., 1983; Owada et al., unpubl.).

Materials and methods

The surface water of the head of a reservoir, in Miyazaki Prefecture, Japan, was taken on November 28, 1989. The sample contained only P. bipes, when examined microscopically, and its population density was $3.6 \times 10^4$ ml$^{-1}$. Almost all the cells were moving actively. Fifty ml of the sample was dispensed into petridishes with an 85 mm diameter. The petridishes were placed under UV lamps which were fixed to the ceiling of an incubator (LH 200 RD, Nihon Ikakiki Co., Ltd, Japan). The strength of UV radiation was adjusted to 4400, 2400, 1500, 700, 390, and 80 $\mu$W cm$^{-2}$ by placing petridishes at different distances from the UV lamp. The intensity of UV radiation was measured by a radiometer (Model UVX Digital Radiometer, UVP Inc., USA). An UV lamp (GL-15, Toshiba Co., Ltd, Japan) emitted 253.7 nm. The culture was carried out under the condition of a continuous illumination of 3000 lux by fluorescent lamp (FL 40 SD, NEC Co., Ltd, Japan) and at a temperature of 15°C. A control experiment without UV radiation was carried out in an incubator without UV lamps.

The changes in the number of dead cells were surveyed under continuous UV radiation until all the cells died and after certain periods of UV radiation of 1 min, 15 min, 30 min, 1 hr, 2 hr and 5 hr. After mixing sample well in a petridish, one ml of sample was transferred into a 1 mm deep counting chamber of 50 x 20 mm$^2$ meshed with a 1mm$^2$ and one drop of Evans blue solution was added with a final concentration of 0.05 % (Satoh and Yamaguchi, 1988). All the live and dead cells in 10 meshes, which were randomly chosen, were counted microscopically. Counting which was repeated 5 times in this way using the same sample gave 2.5 % of a coefficient deviation of s/x x 100 (s:standard deviation of the sample, $\bar{x}$: mean value of the sample). Discrimination of dead cells from live cells was done according to the colour of cells. Dead cells stain pale blue and live cells stain dark blue. Death rates were expressed by the ratio of dead cells to the total cells. The number of dead cells were obtained by subtraction of the number of dead cells of the control experiments from those of each experiment.

Results and discussion

The death rates under continuous UV radiation of each strength are shown in Fig. 1. Continuous irradiation at an intensity of more than 390 $\mu$W cm$^{-2}$ induced death at a ratio of larger than 50 % within 12 hr and that at an intensity of more than 80 $\mu$W cm$^{-2}$ killed all the cells within 48 hr. As the UV radiation becomes stronger, the pattern of the death rate curve changed from linear to logistic. The relationship between the strength of
UV radiation (W) and the time required for 50% of the cells to die (TH) is shown in Fig. 2. The relation was expressed by a hyperbolic curve as follows:

\[(\text{TH} - \text{a}) \times \text{W} = \text{c}\]

where a and c are constants.

Therefore, energy consumption of UV which is expressed by W x TH during the period when 50% of the cells dies is proportional to the strength of UV radiation.

The relationship between the strength UV radiation and the time required for all the cells to die (TA) is shown in Fig. 3. All the cells died by 6 hrs and 48 hrs under UV radiation greater than 2400 and 80 \(\mu\text{W cm}^{-2}\), respectively. The relation between W and TA is also expressed by a hyperbolic curve. Therefore, energy consumption of UV which is expressed by W x TA until all the cells die is proportional to the strength of UV radiation as mentioned above.

The death rates after definite periods of continuous UV radiation (T) of each strength are shown in Fig. 4. As seen in the death rates under continuous UV radiation, it was observed that as the UV radiation becomes strong under a certain period of UV radiation, the pattern of the death rate curve changed from linear to logistic. The relationship between the strength of UV radiation and the time required for 50% of the cells to die after the end of a certain period of UV radiation (TH') is shwon in Fig. 5. UV radiation stronger than 700 \(\mu\text{W cm}^{-2}\) for one min killed 50% of the live cells within 15 days. As shown in Fig. 4, UV radiations of 80 and 390 \(\mu\text{W cm}^{-2}\) for one min had no effect on the decrease in the number of live cells. However, UV radiation of 80 \(\mu\text{W cm}^{-2}\) longer than 30 min killed 50% of the live cells within 7.7 days. The relationship between W and TH' under different periods of UV exposure was described by a hyperbolic curve.
Fig. 4. The death rates after certain definite periods of each strength continuous UV radiation (□, 80 μW cm$^{-2}$; △, 390 μW cm$^{-2}$; ○, 700 μW cm$^{-2}$; ■, 1500 μW cm$^{-2}$; ▲, 2400 μW cm$^{-2}$; ●, 4400 μW cm$^{-2}$). Numbers in the graphs indicate periods (min) of UV radiation.
The time for 50% of the cells to die after the end of the certain periods (○, 1 min; △, 15 min; ■, 30 min; ○, 1 hr; △, 2 hr; □, 5 hr) of each strength of UV radiation.

The relationship between the strength of UV radiation and the time required for all the cells to die after the end of a certain period of UV radiation (Tₐ) is shown in Fig. 6. The relationship between W and Tₐ under different periods of UV exposure was described by a hyperbolic curve except in the case of one min radiation. This indicates there is a minimum strength of UV radiation required for a certain period of exposure to kill all the live cells. All the live cells were killed by UV radiation of 80 µW cm⁻² for longer than 2 hr, 390 µW cm⁻² for longer than 1 hr, 700 µW cm⁻² for longer than 15 min, 1500 µW cm⁻² for longer than 15 min 2400 µW cm⁻² for longer than 1 min and 4400 µW cm⁻² for longer than 1 min within 6, 8, 8, 3, 19 and 11 days, respectively. Therefore, the least energy to produce UV radiation, which is expressed by W x T, to kill all the live cells is in the case of 2400 µW cm⁻² for one min.

As seen above, UV radiation effectively killed live cells. However, when the UV radiation is practically applied to elimination of P. bipes, in a reservoir, the effectiveness depends on the apparatus for UV emission, the way of operation and the distribution pattern of the organisms. Furthermore, it must be considered for the dead cells not to induce a secondary pollution by recycling of nutrients through decomposition of dead cells.

Fig. 6. The time for all the cells to die after the end of a certain period (○, 1 min; △, 15 min; ■, 30 min; ○, 1 hr; △, 2 hr; □, 5 hr) of each strength of UV radiation.
Reference


